Psychological Monographs

General and Applied

Water Regulation in the Rat:

III. The Artificial Control of Thirst with Stomach Loads of Water and Sodium Chloride

By

Lawrence I. O'Kelly and
Robert C. Beck

University of Illinois

Price $1.00

Edited by Norman L. Munn

Published by the American Psychological Association, Inc.

Psychological Monographs:

General and Applied

Combining the *Applied Psychology Monographs* and the *Archives of Psychology* with the *Psychological Monographs*

NORMAN L. MUNN, Editor

Department of Psychology, Bowdoin College
Brunswick, Maine

Manuscripts and correspondence on editorial matters should be sent to the Editor. *Psychological Monographs* publishes comprehensive experimental investigations and other psychological studies which do not lend themselves to adequate presentation as journal articles. Major space is given to the author's original contribution; introductory and bibliographic materials, as well as statistical tables and graphs, must be kept within reasonable bounds. Tables, graphs, and appendix materials which deal with detail not essential to adequate presentation of the findings may be made available through the American Documentation Institute—for details of this procedure, see the APA *Publication Manual.* Preparation of manuscripts for publication as monographs should follow the procedure given in the APA *Publication Manual.* Publication in *Psychological Monographs* is free of cost to the author, except in cases where early publication is requested or author's alterations are made in galley proofs.

ARTHUR C. HOFFMAN
Managing Editor

HELEN ORR
Promotion Manager

Correspondence on business matters should be addressed to the American Psychological Association, Inc., 1333 Sixteenth St., N.W., Washington 6, D.C. Address changes must arrive by the 10th of the month to take effect the following month. Undelivered copies resulting from address changes will not be replaced; subscribers should notify the post office that they will guarantee third-class forwarding postage.

Vol. 74, No. 13 Whole No. 500, 1960

Psychological Monographs: General and Applied

WATER REGULATION IN THE RAT:

III. THE ARTIFICIAL CONTROL OF THIRST WITH STOMACH LOADS OF WATER AND SODIUM CHLORIDE[1]

LAWRENCE I. O'KELLY AND ROBERT C. BECK[2]

University of Illinois

ALTHOUGH the study of motivational variables in relation to other behavioral phenomena has become a matter of increasing interest, the actual number of ways in which motivation can be controlled is indeed small. In animal experiments under hunger or thirst motivation, procedures have been limited almost exclusively to variations in the techniques of deprivation. A brief description of some of the variants of the "natural" procedure will serve to clarify the need for additional methods of controlling motivational variables. The purpose of this monograph is (*a*) to describe a procedure for "artificially" manipulating the thirst drive of rats by the placement of stomach loads of varying volume and composition, and (*b*) to present a series of standardizing, parametric studies of some of the principle variables involved.

THIRST CONTROL

Natural Methods

Subjects maintained on deprivation schedules differing in duration. The shortcomings of this procedure are several. Under differing hours of water deprivation the *S*s have different degrees of associated hunger, it having been shown that food intake is less when water is absent from the animal's living quarters. Thus, thirst *and* hunger effects are confounded. Further, if, having maintained *S*s on a particular deprivation schedule, one wishes to shift them abruptly to a different schedule, the *S*s may turn out to be somewhat less flexible than the experimenter. The animal adapts to particular maintenance schedules only gradually, and changes in behavior following schedule disruption cannot be attributed solely to changes in "drive strength," for example. Differences in duration of deprivation often must be quite large in order to produce behavioral differences of measurable amount. This means that *S*s may have to be run at irregular times which may fall in different parts of the same or different diurnal cycles, a factor which is usually reflected in greatly increased performance variability. Another serious hazard of deprivational methods arises when they are used for producing drives of high intensity. Long deprivation periods (longer than 36 hours of water lack) inevitably result in debilitation and inanition when continued long enough to be of any experimental value, thus introducing another confounding factor. For example, in situations where performance declines from maxima achieved at intermediate deprivational levels, it has always been difficult to determine whether drive increases continuously with hours of deprivation.

Predrinking. Sometimes all *S*s are maintained on a common schedule, but are allowed to drink different amounts of water prior to testing or training. This, however, has the disadvantage that differential predrinking itself is a variable affecting per-

[1] This series of experiments was partially supported by grants from the National Science Foundation and from the Graduate College of the University of Illinois.

Appreciation is expressed to Emanual Lask, Burton Slotnick, and Yasuko Azuma, who assisted in various stages of collecting and analyzing data.

[2] United States Public Health Service Postdoctoral Fellow. Now at Wake Forest College, Winston-Salem, North Carolina.

formance (Bruce, 1937, 1938; Miller, Sampliner, & Woodrow, 1957). Moreover, if an investigator wishes one of several groups to be maintained on a long or severe deprivation schedule, then all *S*s must be maintained on that schedule, with the consequent health risks as noted above.

Running at varying times after drinking. A variation of the above procedure is to maintain all *S*s on the same schedule, but run them at varying intervals after drinking. This has some advantage over a predrinking method, but still does not solve the differential hunger problem since *S*s typically eat just after they have drunk. One can readily shift hours of deprivation simply by running the *S*s at different times after drinking; but if the *S*s have learned to perform at a certain time it is difficult to know whether to interpret the change in performance as being due to differences in drive per se or to the changes in the running schedule.

Maintenance at a percentage of body weight. This deprivation is commonly used with both rats and pigeons for the control of hunger, but does not appear to be well-suited to control of thirst. Whereas a 10-20% weight reduction with food deprivation makes a good active animal for much research, such a water loss could not be tolerated by the rat. Most important, perhaps, is the fact that the effects of a weight-controlling procedure would inevitably be confounded with the effects of the accompanying reduced food intake; so that the loss could not appropriately be attributed to water deficit alone, and the water loss itself could not be differentially estimated.

Artificially Controlled Thirst

Because of the many disadvantages and inconveniences attached to control of thirst solely through duration of water deprivation, we have expended considerable effort on the study of alternative ways of accomplishing the same ends. There are a number of very clear advantages of the artificial methods. All *S*s can be maintained under comparable living schedules, irrespective of any differences in the motivational states they are under during the actual experimental observations. A variety of intensities of thirst can even be induced in the *same* animal on different occasions without change of the daily maintenance schedule. An earlier study (O'Kelly, 1954) showed that the effects of water loads are independent of each other if there is a two-day recovery period between loads; in the experiments to be reported in this paper, the same is true for a variety of NaCl loads, although the length of the recovery period is slightly longer.

Another important advantage of artificial control is that variations in thirst can be obtained and sustained independently of variations in consummatory activity, thus permitting the study of relationships between various parameters of tissue need and behavior without the complications introduced by behavioral consequences of the consummatory activity itself. Whether physiological imbalances vary concomitantly with *drive* is still an important unanswered question (O'Kelly & Heyer, 1951; Towbin, 1949). Wayner and Reimanis (1958) found that running speed increased as a function of time after hypertonic salt injections, but that the amount of water consumed remained constant. Experiments of this type illustrate the advantages of being able to influence separately those variables of tissue disequilibrium from the temporal and spatial supports with which they are inevitably associated in natural deprivation.

Methods of Control

It is well known that drinking can be stimulated artificially by introducing into the animal's blood stream a number of solutions of concentration hypertonic to that of the plasma (i.e., having an effective osmotic pressure greater than that of the plasma). Sodium chloride has been the substance of choice, since, as the normal major extracellular electrolyte, it produces the best combination of drinking enhancement with minimal toxic or other side effects. The solution may be delivered to the plasma by

a variety of routes: intravenously, intraperitoneally, or subcutaneously by injection; directly into the hypothalamus or third ventricle by stereotactically placed hypodermic needles; or, finally, introduced into the digestive tract by fistula or esophageal tubing. Since the present monograph is concerned only with use of the stomach loading approach, we will limit our comments on the other techniques to those results that have stemmed from our own previous trials of the subcutaneous injection of saline.

Subcutaneous injection. Proceeding from the findings of Gilman (1937) and of Holmes and Gregersen (1950) that intravenous injections of hypertonic sodium chloride solution increased water intake, Heyer (1951) showed that similar enhancement of drinking in the rat could be induced by subcutaneous injections of 15% NaCl solutions. Further, by varying the amount of NaCl injected, water intakes equivalent to those observed after 12, 24, or 36 hours of water deprivation could be secured. Further standardization of the injection technique was done by Wayner (1953) and Wayner and Reimanis (1958). While drinking can be potentiated by this means, certain disadvantages are characteristic of the procedure. The most serious is the skin damage and pain to animals that may accompany too long a series of repeated injections. This limits the number of days over which unconfounded observations may be made. Also, by the nature of the treatment, it is practicable only to *enhance* drinking from any base reference level, whereas an ideal technique of drive manipulation should permit controlled variation from satiation on up (and possibly down, in the direction of water excess). While drinking potentiation is a function of the total amount of salt, rather than its concentration, drinking inhibition is a function of volume; subcutaneous injections of volumes of sufficient size to limit materially the subsequent water intake would be impracticable to administer.

Stomach loading. For use with rats or other animals of a similar size, it would appear that loading of fluids directly into the stomach via esophageal tubing is the better method. The rat's stomach will accommodate relatively large volumes, thus giving the experimenter a greater latitude of choice in both volume and concentration of administered fluid. Since the rat has no regurgitatory reflex, fluid is not lost during or after placement, as is often the case with hypodermic injection. Also, quite importantly, the route through stomach and intestine is the usual path taken by ingested fluids, and the various steps along the way from stomach to ultimate arrival in the extracellular fluids will more nearly involve normal physiological processes and normal latencies of action.

Administration of fluids in this manner appears to be relatively painless, and it is a matter of usual observation that animals do not develop avoidance habits in relation to the experimenter or the general laboratory situation. As the technique is used in our laboratory, the rats are lightly etherized before loading. This reduces struggling, and the procedure can easily be accomplished with a minimum of difficulty by one person. The time for etherization and loading normally range from 1.5 to 2 minutes. General recovery from the anesthetic is rapid, the animals appearing grossly normal within 5 minutes. There is a small but reliable depression of drinking as a result of exposure to ether (O'Kelly & Weiss, 1955), but this does not appear to interact in any specific manner with load effects.

Previous studies using the stomach loading technique have demonstrated that (*a*) water consumption by 23.5-hour water-deprived rats can be depressed in quantitative fashion by varying volumes of water loads, or can be enhanced by hypertonic NaCl loads (O'Kelly, 1954); (*b*) bar pressing may be similarly enhanced or depressed by such loads (O'Kelly & Falk, 1958); and (*c*) runway performance is affected in a like manner (Solarz, 1958). Thus, the technique is capable of producing behavioral effects consistent with the manipulated need state. Finally, water regulation has been studied in some detail by means of this and related procedures so that such physiological variables as rate of clearance of fluids from the stomach and

their intestinal absorption following loading are partially understood (O'Kelly & Falk, 1958).

The studies presented in this monograph are a continuation of the program cited above. They constitute a further extension of our efforts to quantify the control of thirst in the rat by means of stomach loading technique, and to study relations between the need state of tissue dehydration and resulting behavior.

Outline of General Procedure

With the exception of various details of management of experimental variables, the procedure used in the drinking studies (Experiments 1-4) was the same for all, and can be outlined first.

Subjects. The *Ss* in Experiments 1–4 were experimentally naive male albino rats of the Holtzman strain, from 100 to 130 days of age at the start of the experiments. They had Purina laboratory chow constantly present in their home cages during all phases of the experiments, but once an experiment was started they did not receive water in the home cages until it had been completed.

Adaptation and recovery periods. Prior to any experimental treatment the *Ss* were put on a 23.5-hr. water deprivation schedule for 10 days. Each day they were brought from the home cages to the laboratory where they were weighed and then given access to water for a period of 30 min. in individual drinking boxes. On load days they were weighed at the regular time, but drank in special cages, described below. On all interload days, and for a number of days after the final load day, they were weighed and watered just as during adaptation. Thus a check was maintained on the effects of the schedule and treatments, and the general state of their health was closely followed.

Apparatus. The apparatus consisted of drinking boxes, metabolism cages, and water tubes. The drinking boxes, 4 in. by 11 in. by 6 in. high were constructed of wood and had hinged wire tops. The metabolism cages, circular in shape and made of hardware cloth, were 6 in. high, 8 in. in diameter, and rested on double thicknesses of fine copper wire screen set in the mouths of glass funnels. Underneath each funnel was a 50-cc. centrifuge tube for the collection of urine, the feces being screened out. Funnels and screens were cleaned before each session to insure uncontaminated urine. The drinking tubes were 100-cc. gas-measuring tubes, graduated to 0.2 cc., into which were placed drawn glass drinking spouts. The ends of the drinking tubes were inserted into the drinking boxes and metabolism cages about 1 in. above floor level.

Solutions and drinking water. All saline solutions were made with distilled water, using a weight/volume ratio (Pfaffman, Young, Detheir, Richter, & Stellar, 1954). All loads were given at room temperature. All drinking water was local tap water, given at room temperature. Chloride concentration of the load solutions was checked by chemical analysis.

Load day procedure. On load days the animals were weighed at the regular drinking time and the exact volume of load was determined for each animal. Each *S* was then anesthetized lightly by placing it in a museum jar saturated with ether fumes and given its load by stomach tube (urinary catheter), as has been previously described (O'Kelly, 1954). It was then put into its metabolism cage while it recovered from the ether. A 15-min. delay period was used in all the drinking experiments except 4, which was specifically a study of the effects of delay of access to water after loading. At the end of the delay period the water tube was inserted into the cage and water intake and urine output were recorded at 5-min. intervals for 30 min., 15-min. intervals for the next 90 min., and at 30-min. intervals for the final 120 min., a total of 4 hr. At the end of 2 hr. the centrifuge tubes collecting the urine were changed so that separate urinalyses could be made on the first and last halves of the drinking period.

Urinalyses. Immediately following their collection, all urine samples were put into screw-cap vials and refrigerated until they could be analyzed. In the first two experiments analyses were made for chloride content, using the method of Schales and Schales (1941). In the later drinking experiments sodium and potassium content were determined as well, using a Beckman flame photometer.

Experiments 1 and 2: The Effects of Water and Sodium Chloride Loads on Thirsty and Satiated Rats[3]

Method

Subjects and experimental design, Experiment 1. Sixty rats were used, averaging 361 grams in weight on the first load day. The experiment was designed specifically to study the effects of five different volumes of hypertonic loads and water on the 4-hr. intake of 23.5-hr. deprived rats.

[3] R. C. Beck and L. I. O'Kelly. Since the responsibility for design and execution of the various parts of this series varies from experiment to experiment, authorship will be indicated in this fashion.

TABLE 1

VOLUMES, CONCENTRATIONS, AND ABSOLUTE NaCl CONTENT OF LOAD SOLUTIONS

Volume % of Body Weight	NaCl Concentration (grams/litre)						
1	0[a]	3.0	*5.8*	*9.1*	*12.0*	*15.3*	*18.5*
2	0		*3.0*	*4.5*	*5.8*	*7.4*	*9.1*
3	0		*2.0*	*3.0*	*3.9*	*4.9*	*5.8*
4	0		1.5	2.3	3.0	3.6	4.5
5	0		1.2	1.7	2.4	3.0	3.6
	Isosals (grams NaCl/kilogram body weight)[b]						
		0.3	0.6	0.9	1.2	1.5	1.8

Note.—All loads were used in Experiment 1. Italicized loads were used in Experiment 2.

[a] "0" indicates tap water load.

[b] Due to slight errors in mixing solutions the isosals are medians for the five different volumes of load.

There were five different NaCl concentrations at each volume. The treatments are summarized in Table 1.

Three characteristics of Table 1 should be pointed out. First, the concentrations at each volume were selected so that the highest concentration would approach a near-lethal dose and so that there would be equal steps between the concentrations at a given volume. The differences depart slightly from equality due to slight variability in mixing the solutions. A lethal dose was estimated as being an absolute amount of salt equal to about two grams per kilogram of body weight. This estimate was based on extrapolation from results in preliminary studies which had included a few actual lethal doses.

Secondly, each of the five concentrations at a given volume represent the same absolute amount of salt as are found in the concentrations at any other load volume. Consequently, we have two ways of studying the effects of concentration of solution on drinking: (*a*) by varying the volume of load and holding the absolute amount of salt constant (reading down each of the columns in Table 1); or, (*b*) by holding the volume constant and varying the amount of salt (reading across each of the rows in Table 1). A given amount of NaCl, in varying volumes of solution, is referred to throughout this paper as an *isosal.* There are in this experiment five *isosals,* the tap water loads not being considered as such.

Finally, the 3.0% concentration was included at the 1% volume so that this concentration would appear with all volumes. It alone does not have an isosal counterpart at all volumes.

The 60 *S*s were divided into two groups of 30, making two simultaneously run replications of the experiment, one in the morning and the other in the afternoon. Each *S* received a different load on each of three separate days, with a four-day recovery period between loads. Treatments were randomly assigned to animals with the restrictions that (*a*) no *S* received the same load twice, and (*b*) each treatment occurred equally often on first, second, and third load days. There was a slight departure from the last restriction in that there were five, rather than six, observations for six of the 31 treatments. Because it was impossible to load and observe more than 10 animals in a half-day, the experiment was staggered, each treatment "day" being divided into three separate daily sessions, with 10 animals loaded in each session.

Subjects and experimental design, Experiment 2. Whereas the first experiment used *S*s 23.5-hr. water-deprived at the time of loading, this study dealt with the effects of such loads on "satiated" rats, i.e., rats in a state of water balance at the time of loading. Since it was deemed desirable to maintain *S*s on a drinking schedule, satiation was achieved on load days by giving them their regular 30-min. drinking period. They were then removed to a common retaining cage and following a 1-hr. delay, a time previously shown to be sufficient for water to clear the stomach and be absorbed from the intestine (O'Kelly, Falk, & Flint, 1958), were loaded and given their 4-hr. drinking period.

Thirty rats were used, weighing an average of 369 grams on the first load day, about 8 grams heavier than the *S*s in Experiment 1. The only difference in design from Experiment 1 was that the hypertonic loads were given at only three volumes (1, 2, and 3% body weight) and no water loads were given. The volumes and concentrations used are those in italics in Table 1. Since each animal was loaded three times, there was a total of 90 loads, 6 *S*s for each of 15 treatments.

Results of Experiments 1 and 2

In order to facilitate comparisons, the results of both experiments are presented simultaneously. Before doing so, however, it is necessary to comment on the statistics. Unless otherwise stated the analyses were three-way analyses of variance: load concentration × load volume × day of loading (first, second, or third). Since the number of *S*s having any particular sequence of treatments was too small to be analyzed, it was assumed that each *S*'s three different treatments were independent of each other, and that the number of independent obser-

vations was equal to the number of loads given. In Experiment 1 only the treatments including the five isosals common to all volumes were used in the analyses; water loads were excluded. There being no differences between replications, they were combined. All the treatments in Experiment 2 were included in the analysis. Only significant F ratios are reported, these being given at appropriate places in the text.

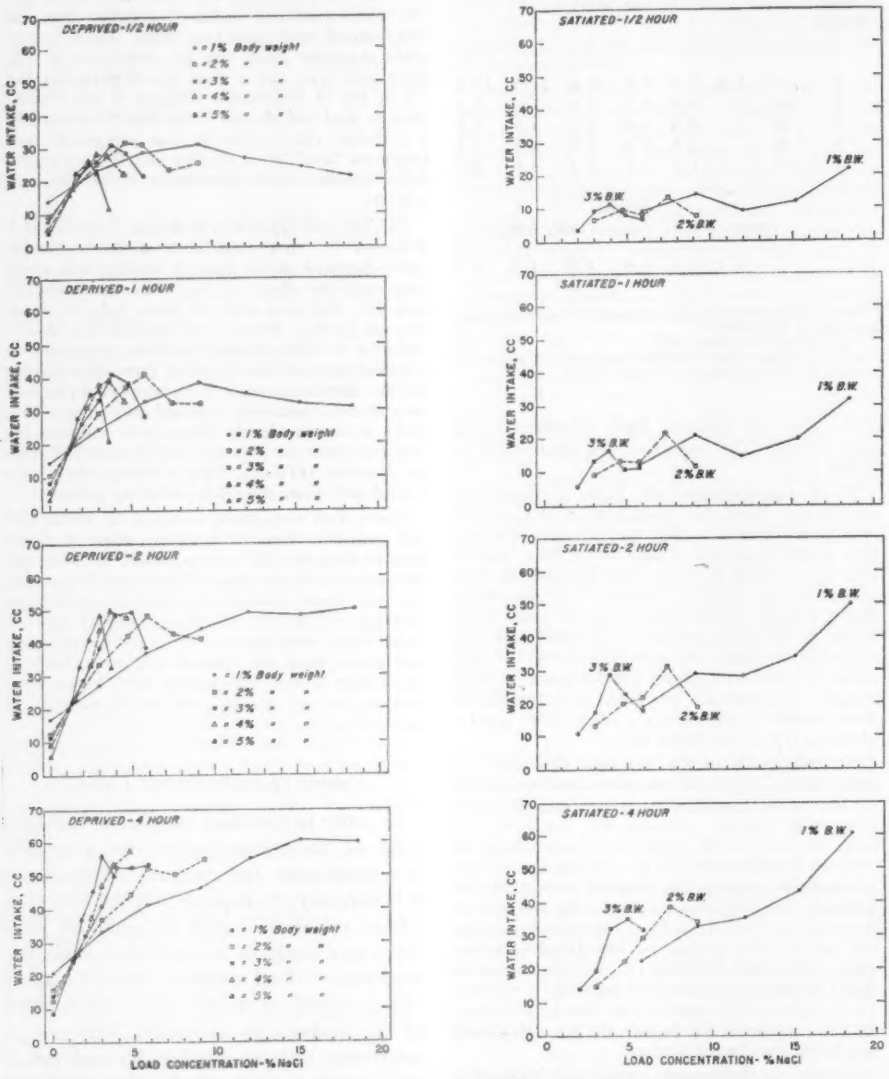

FIG. 1. Water intake in Experiments 1 (deprived) and 2 (satiated) as a function of load volume and concentration at four intervals after access to water; ½, 1, 2, and 4 hours. (Intakes of 23.5-hr. deprived control animals—no experimental treatment—are 19.2, 19.7, 21.2, and 24.7 cc. for these same intervals.)

Water Intake

Figure 1 summarizes the 30-, 60-, 120-, and 240-minute water intakes as a function of volume and concentration of load. For the deprived animals, in the early part of the drinking period (30-60 minutes) drinking increases in a near-linear fashion with load concentration up to some optimal point of concentration, and then declines. With increasing volumes the optima occur with progressively smaller concentrations and in fact occur at each volume with approximately the same amount of salt, between 0.9 and 1.2 grams per kilogram of body weight. The exact optimal salt load is indeterminate from these data, but data from another experiment indicate that the optimal concentration for 2% volume is about 5%, or 1 gram/kilogram of body weight.

The form of the 30-minute intake curves differs between the deprived and satiated *Ss*. The 3% volume curve is similar in the deprived and the satiated groups, but the 2% deviates at the 5.8% concentration, which is lower than the 4.5% when it is expected to be higher. The 1% volume curve shows little patterning at 30 minutes. Individual drinking curves show that the reason for this discrepancy between the satiated and deprived groups is the ability of some of the satiated *S*s to go for long periods without ingesting water. For example, one *S* given a 3% volume of 6% NaCl (the maximum volume and concentration in Experiment 2) drank nothing in four hours. A number of other animals failed to drink in two or three hours.

Total intake for four hours tends to be proportional to the amount of salt loaded in both experiments, except that the most concentrated loads at any volume produced an inversion in this amount of time. With drinking periods longer than four hours, presumably all would be monotonic functions.

Separate analyses of variance were calculated in each experiment for intake during the first two hours, second two hours, and for the total four hours. The results were essentially the same for both experiments, except that there was a significant day effect in Experiment 1, the total mean intakes being 43.7, 46.5, and 50.1 cc. on the successive load days. (This effect is studied more exhaustively in Experiment 3.) The most potent influence was the amount of salt in the load, with volume playing a much smaller role, and with almost no interaction between the three variables. With increasing load volumes there was a corresponding decline in drinking, presumably due to the extra water included in the larger loads, a circumstance requiring the animals to take in a lesser fraction of their water by drinking.

Further analyses were computed for the two experiments jointly, using the 1, 2, and 3% volumes from Experiment 1. The deprivation effect was highly significant during the first two hours ($F = 130.96$, but not during the second two hours ($F < 1.00$). When the amount predrunk by the satiated animals was added to their first two-hour intake, however, the F value for deprivation was reduced literally to zero. The average four-hour intake for the deprived animals at the 1, 2, and 3% volumes was 48.80 cc.; that of the satiated animals, with predrink added, was 47.56 cc. It seems clear that the deprived animals drink first of all to offset their 23.5-hour deficit, then to counteract the salt loads.

The initial high intake rate of the deprived animals as compared to the satiated is demonstrated clearly in Figure 2, which shows the rate of intake over four hours as a function of isosals, with all load volumes combined. These curves, though somewhat stylized due to combining, still reflect accurately the individual volume curves. For the deprived animals, at all volumes, the initial depression of drinking due to high concentration loads is gradually made up throughout the four hours, whereas at the lower concentrations the animals take in their water quickly (30-60 minutes) and drink very little thereafter. The satiated animals drink at a slower rate in the first hour than do the deprived, but with the higher isosal loads they, too, drink steadily throughout the four hours.

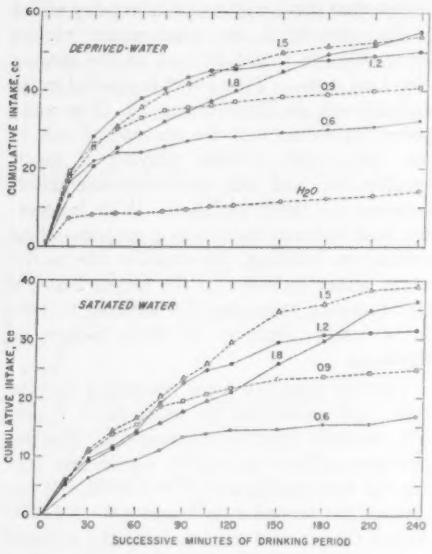

FIG. 2. Four-hour drinking rates as a function of isosal in Experiments 1 (deprived) and 2 (satiated). (Time zero on the abscissa is when the animals were given access to water after loading. For the H_2O loads and each isosal, the rates for all load volumes have been combined.)

Figure 3, from data in Experiment 1, shows the rate curves for five volumes of H_2O load and the five volumes of 3% NaCl load. Water intake following H_2O loading decreases with the magnitude of the load over the entire four hours, but the rates for varying volumes of 3% NaCl show the same general trend as the data in Figure 2, with an increase in intake up to an optimal point during the early part of the drinking period, then a depression with higher volume. At the end of four hours intake is essentially a linear function of load volume. These results give further indication that the amount of salt in the load is the major determiner of intake.

Urine Output

Figure 4 shows the rate of urine output for the deprived and satiated *S*s. As with water intake, the most potent determiner of urine volume is the amount of salt in the load, F values of 64.23 and 24.74 being obtained in the two experiments for four-hour urine output. The F values for the variable of load volume, on the other hand, were 7.70 (less than .01) in Experiment 1 and 2.32 (*ns*) for Experiment 2. The day effect was not significant in either experiment.

In Experiment 1, urine volume increased with load volume up to 4% body weight during the first two hours, but declined at 5%. For the total four hours, on the other hand, the increase was monotonic with load volume. In Experiment 2, both two-hour and four-hour urine volumes increased from 1% to 2% load volumes, then declined at 3% load volumes.

Generally, however, the most striking effect with respect to urine volume is the almost complete lack of difference between the two experiments. A combined analysis of variance showed that while the satiated animals did excrete a statistically greater amount ($p = .05$) than the deprived during the second two hours, there was no difference for the first two hours or total four

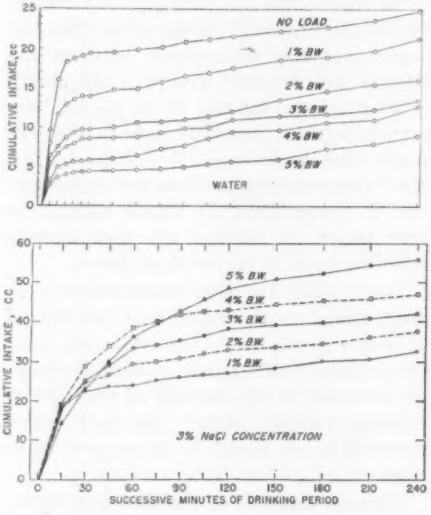

FIG. 3. Four-hour drinking rates as a function of load volume following H_2O loads (upper) and 3% NaCl loads (lower). (All data were obtained from Experiment 1, the number of *S*s for each curve being six.)

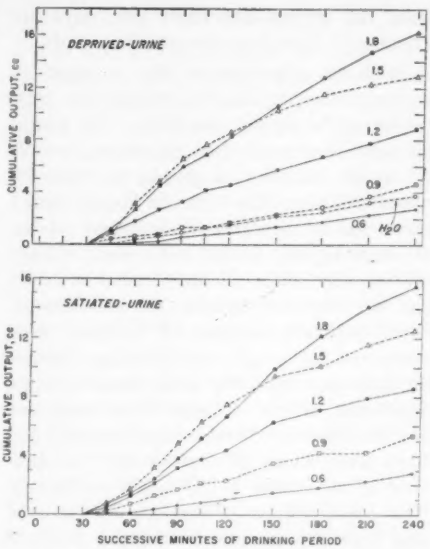

FIG. 4. Four-hour urine rates for Experiments 1 and 2 as a function of isosal. (The initial higher rate with the 1.5 gm/kgm isosal than with the 1.8 appears to be due to the animals with the 1.5 load having a diarrheic load effect earlier than those with the 1.8 load. In Experiment 1 the antidiuretic effect of low concentration loads is seen where the 0.6 isosal load is followed by lesser urination than the H_2O load.)

hours. The predrink and hour's delay between predrink and loading of the satiated animals evidently does not influence delay of onset of urination after loading, rate of output, or total urine flow.

We find then, with respect to both water intake and urine volume that there are no differences in the deprived and satiated groups other than the extra water needed by the deprived animals to offset their previously incurred 23.5-hour deficit.

The upper part of Figure 5 shows the urinary chloride concentrations for both experiments as a function of isosal. Since urines were collected separately for the first two hours and second two hours, this "half" variance was included in the analysis. Once again, the isosal effect is very large, and in Experiment 2 it is the only significant variance. An unexpected occurrence was the highly significant difference between halves in Experiment 1, where the second-half urine is much more dilute than that from the first half. This effect did not occur in Experiment 2, however, and a combined analysis of variance for the two experiments shows an F value of 24.86 between deprived and satiated in the second half.

The animals have two urinary means of ridding themselves of the imposed NaCl load. They can excrete a larger volume of more dilute urine or a smaller volume of more concentrated urine. As Figure 5 shows, the animals have a fairly constant increase in total urine volume, as a function of isosal, over the four-hour period. Hence this mechanism of chloride excretion (volume) is being used throughout the entire range of isosals. The concentration of urine, on the other hand, increases only up to the second or third isosal, then remains at a level of about 2% (although individual animals were able to concentrate their urine as high as 3%). The lower part of Figure 5 shows the product of concentration and

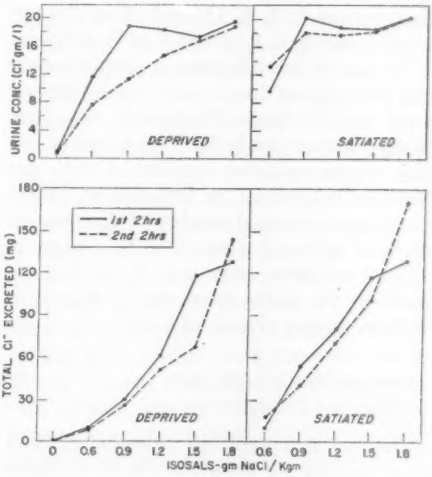

FIG. 5. Urine concentration in grams of chloride per liter (upper), and total milligrams of chloride excreted (lower) as a function of isosal in Experiments 1 and 2.

volume, the total amount of chloride excreted, in milligrams. Whereas the rats may have reached an upper concentration threshold in both halves of the drinking period, they apparently had not reached a volume limit, nor a limit for chloride excretion in the second half. Otherwise, there would have been some asymptotic limit approached for chloride output in the second two hours, such as is indicated in the first two hours. Apparently, there could have been a still greater volume of urine produced had there been more concentrated loads given. Load concentrations above those in this experiment run dangerously close to lethal dosages, however, so it may be that urine volume would increase right up to the final lethal dose.

In relation to the usefulness of various loads for behavioral experiments, it may be noted that the occurrence of diarrhea following loading is of some practical interest. Diarrhea occurred commonly at the two highest isosals in Experiment 1 (about 40% of the cases), much more frequently than in Experiment 2 probably due to the presence of more water within the intestinal lumen. The number of cases, by increasing isosals, was 0, 3, 6, 13, and 12 in Experiment 1 and 0, 0, 2, 4, and 1 in Experiment 2. A test of the difference in proportions at the two highest isosals was significant beyond the .01 level. Using only the three lowest volume loads in Experiment 1 for this comparison, the significance level was between .05 and .10. In Experiment 1 there were about an equal number of diarrheas at all load volumes (range: 6-9), except at 1% where there were only 2. In Experiment 2, the distribution was 2, 4, and 1, with increasing volume of load.

Interload Day Weight and Drinking Changes

Weight and drinking changes on days after loading are shown in Figure 6. Since there was no regular drinking period on load days in Experiment 1, the day before loading was taken as the baseline. The satiated animals have an extra recovery day since the second and third load days are "recovery" days from the previous loads.

In both experiments the increase in weight following loading reflects the total intake on the previous load day. The weight changes increased with increasing isosals up to the fourth (1.5 gm/kgm), then declined sharply at the fifth. In Experiment 1 there was an overall correlation of .51 between load day intake and weight change on the day after. Water loads, however, had no effect on weight. In each experiment, separate analyses of variance were computed for weight and drinking changes on each post-load day until there were no significant effects. *T* tests were computed for the difference between baseline days and days after, using all observations, irrespective of treatment (H_2O loads excluded). The main difference between the satiated and deprived animals is that the deprived were still significantly overweight at the end of four days, while the satiated animals returned to their preload weights in this period of time. As expected, concentration was the most important variable (a significant effect until the third post-load day), with some small influence of volume. The greater regularity of the curves for deprived *S*s in Figure 6 may simply be a matter of reliability since there were more *S*s in the first experiment, but it more likely corresponds to the greater regularity of intake on load days.

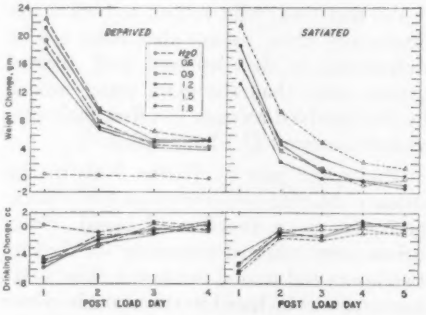

FIG. 6. Weight and drinking changes on days after loading as a function of isosal.

Discussion

The first major result of the present experiment is that drinking, urine volume, and urine concentration are mainly a function of the amount of salt loaded. This agrees with the findings of Wagner and Reimanis (1958) for subcutaneous salt injections. Load volume effects, while significant, are comparatively slight and seem to be due to the fact that, since higher volume loads depart less from isotonicity than small volume loads, they require the animals to drink less water to offset the experimentally-produced deficit.

The second critical result is that salt loads can depress drinking, as well as potentiate it. Thus, whereas the lower concentration loads produced immediate drinking at a high rate (due partly, of course, to the 23.5-hour deficit), the higher concentration loads produce a lower but more persistent rate of drinking. This suggests (with supporting evidence from Experiment 6) that the higher concentration loads are not well-suited for use in behavioral experiments, at least with the 15-minute delay after loading. Direct observation of the stomachs and intestines of animals loaded with high concentration solutions shows them to be very distended, filled with osmotically-induced water from the tissues. Overall activity is considerably reduced. The occurrence of diarrhea serves as a check on this observation.

It would seem a good guess that the highest concentration load practical for the control of behavior would be one at, or slightly below, the optimal load for potentiating drinking in a 30-minute test period (see Figure 1). The loads up to this point tend to show an increment of drinking which is approximately linear and drinking drops off rapidly after about an hour (Figure 2). In other words, these loads seem to produce an immediate change in water deficit which is offset within a minimal time.

The great similarity between the deprived and satiated animals is important with respect to the physiology of salt-load regulation and to the management of behavioral experiments. The deprived *S*s seem to show more regularity than the satiated, however, as shown in their more orderly drinking curves. While larger numbers of animals for the treatments under the two conditions might produce completely analogous drinking results, the lesser variability of the deprived group with only a few animals per condition is just a point in its favor. One of the sources of greater variability for the satiated animals is the fact that they can manage the load either by drinking or by excreting. Some of the satiated animals seemed able to utilize body water very effectively for kidney function during the first part of the drinking period and took in their "environmental water" later in the period. The deprived animals, of course, do not have as much tissue water to expend in kidney regulation at the time they come into the test period.

Experiment 3: The Effects of Repeated Water and NaCl Loads on Thirsty Rats[4]

This experiment was designed to study the cumulative effects of six repeated load treatments, using a six-day intertreatment interval rather than the four-day interval of Experiments 1 and 2. For reasons just discussed, concentration values which were on the accelerated portion of the 30-minute intake curve (Figure 1) were selected. Of the five volumes in Experiment 1, the 2% body weight volume seemed to give the greatest amount of drinking. The concentrations used in this volume did not appear to be such as to overly irritate the digestive tract, and the loads were of small enough volume to be readily tubed into the animals. The decision to study thirsty animals rather than satiated was guided by two considerations: the greater regularity of results with the deprived animals, and the possibility of either enhancing or depressing the drinking from a 23.5-hour deprivation base line.

[4] R. C. Beck and L. I. O'Kelly.

Method

Since the optimal concentration value for a 2% load appeared to be about 6% (in terms of maximal potentiation of drinking), it was decided to use concentrations of 0 (tap water), 2.0, 4.0, and 6.0% NaCl. Initially there were 10 animals in each group, but in the course of the experiment five were lost due to death or treatment error, so that 35 finally completed the experiment. There were nine subjects in three of the groups and eight in the fourth; the weights of all *S*s averaged 363 grams on the first load day. All were male Holtzman albino rats about 100 days old. Following adaptation as previously described, each *S* was given the same load once a week over a period of six weeks. Methods of collecting drinking and urine data were the same as in the previous experiments.

Results and Discussion

Water Intake

Figure 7 shows the rate of water intake for the four different load concentrations on the first load day. We see here that our choice of 6% NaCl as the "optimal" load for potentiating drinking was incorrect, since these animals actually drank less than those loaded with 4% during the early part of the drinking period. As a check on what the "true" optimum might be, a different group of 10 animals was given a 2% volume load of 5% NaCl. These *S*s, in 30 minutes, drank a mean of 37.2 cc., which was above that previously obtained with any load. After four hours of drinking, however, intake (57.9 cc.) was so far above the extrapolation from lower concentrations as to suggest that concentration was not the only variable operating (see Figure 21). It is clear, however, that the optimal load concentration is closer to 5% than to 6% NaCl.

The broken lines in Figure 7 show the rate of intake following H_2O loading on the second through sixth load days. There are no large differences in the first hour or so, but by two hours there is a clear separation of rates, and by two and a half hours intake varies as a function of load day, only the second day being out of order. The results with this particular load concentration are representative of those obtained with the other three concentrations. As Figure 8 shows, total four-hour intake increased equally for all groups over load days.

There was virtually no overlap of individual *S*'s intakes between groups on any given load day. Thus, almost all water-loaded animals drank less than 2% animals, who in turn ingested less than the 4% group, and so on. The differential treatments thus were highly reliable, and there were no observable ill effects to the animals' health occasioned by the repetition of loads.

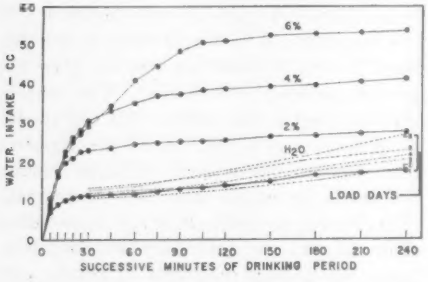

FIG. 7. Cumulative intake for all groups on Load Day 1 (circles) and cumulative intake from 30 minutes on for H_2O loads on Load Days 2 through 6 (dotted lines). (By the end of 4 hr. all H_2O load day intakes are ordered by day: 1-6. The results were similar for the other loads, differing only in absolute values.)

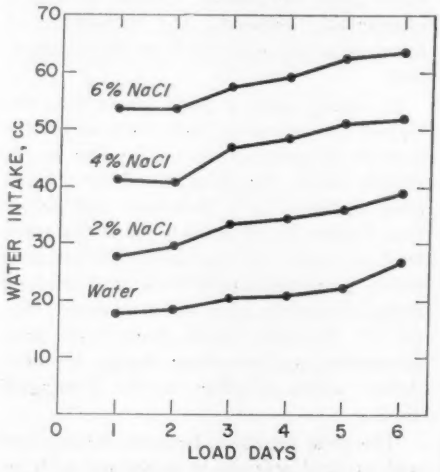

FIG. 8. Four-hour water intake for all groups on successive load days.

The within-group reliability was high also, as indicated by rank-order correlations for total intake from one load day to the next. These correlations averaged between .50 and .70 for adjacent treatment days, and then declined toward zero with an increasing number of treatment days separating the days correlated. Since the groups were small, and were taken from a very homogenous population, relatively minor changes in intake would have a large influence on the size of the correlations. For most purposes the good separation between treatments is the most important consideration. The size of the correlations for successive load days suggests that with larger numbers of *S*s, individual differences could be successfully studied.

Urine Volume and Concentration

The day-to-day changes in four-hour urine output are shown in Figure 9. These changes are of the same sort as occurred with water intake, tending to increase independently of a particular treatment. There is, in fact, a good correlation between intake and urine volume on each load day. The median correlations for the six load days were .74, .70, .25 and .64 for the 0, 2, 4, and 6% loads, respectively.

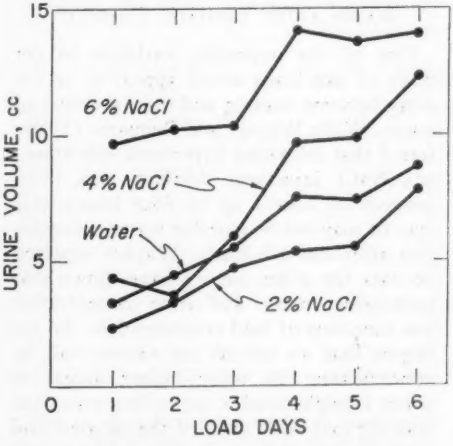

FIG. 9. Four-hour urine volume for all groups on successive load days.

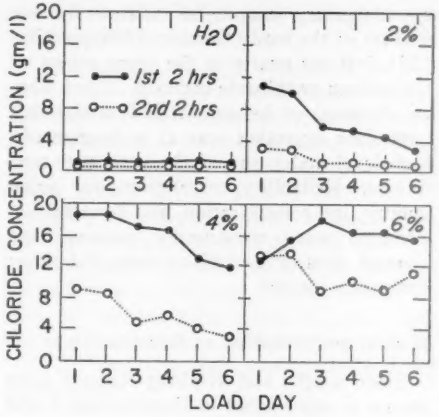

FIG. 10. Chloride concentration during first and second halves of the four-hour drinking period on successive load days.

In Figure 10 the concentrations of urinary chlorides are shown for each of the load days. Whereas chloride concentration tends to decline from day to day, total chloride excreted remains relatively constant or increases slightly. Median chloride excretion in milligrams for the first and last three load days were, for the four groups: 2.3 and 4.0, 14.2 and 13.0, 43.5 and 65.3, and 112.0 and 121.8, respectively. The increase in urine volume offset the decline in concentration of urine, and the net efficiency of chloride excretion remained as great at the end as at the beginning of the experiment. The volume increase in urine parallels the increase in water intake on successive load days, but the direction of causal relationship is not determinable from the present data. It is possible that the repeated dehydration stress of the salt loads, superimposed on a chronic negative water balance maintained by the 23.5-hour deprivation schedule progressively reduces the efficiency of the hormonal regulation of volume excretion, perhaps through exhaustion of antidiuretic hormone. This can only be decided, however, by future experimentation.

Excretion of sodium and potassium through load days was similar to that of chloride. Potassium loss increased as a function of load concentration, presumably

an obligatory output stimulated by the sodium of the load (Gamble, 1958, pp. 126–128), but not nearly to the same extent of the sodium or chloride increase. There were no chemical or behavioral indications that potassium excretion was at a dangerously high level under any of the treatment conditions. Reliability correlations for urine volume, ion concentration, and for total ion excreted were consistently positive, but showed greater variability than did those for water intake.

Weight and Drinking on Interload Days

Since weight and drinking changes were shown in some detail in Experiments 1 and 2, it is sufficient to report that the present experiment confirmed the earlier findings. These changes are a function of load concentration and are quite stable from one load day to another.

Daily water intake returns to normal within two or three days after loading, but weight does not necessarily do so. Figure 11 shows the mean weights of the four groups during adaptation, on the six successive load days, and on the final day of the experiment. Although weights did not return to their previous level after loading, there were no permanent differential effects of the different loads. This is in spite of the fact that the immediate changes varied from a 2- or 3-gram decrement on the day following tap water loads to a 20-gram increment following 6% loads. Since the slope of the weight increment curve over the first few load days is the same as the slope over the last few adaptation days, prior to any treatment, it appears that the weight increases are due to normal growth, and are independent of the influence of our load variables.

The regular 30-minute intake on days prior to each load day was essentially unchanged throughout the course of the experiment, a further indication of good recovery from the loads. Considering all four groups separately on each of the days prior to a loading, and on the final day of the experiment, the mean intake of the groups ranged from 18.4 cc. to 21.9 cc., these means being distributed around a grand mean of 20.4. The mean intake of all animals on the last day of adaptation was 20.06 and on the final day of the experiment it was 20.95 cc. The animals obviously were not drinking appreciably more or less on the deprivation schedule at the end of the experiment than they were at the end of 10 days' adaptation, and intergroup variability was practically nil.

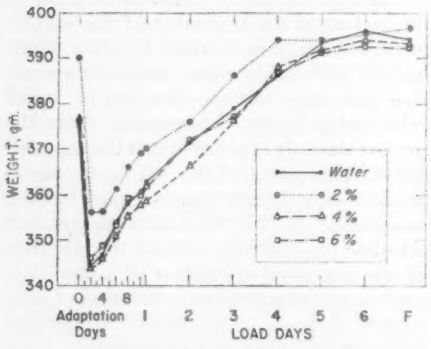

FIG. 11. Mean weights of load groups on alternate adaptation days, each load day, and the final day of the experiment (F). (The interload weight changes—weight increments as a function of load concentration—are not shown.)

EXPERIMENT 4: DELAY OF ACCESS TO WATER AFTER STOMACH LOADING[5]

One of the important variables in the study of salt loads would appear to be the delay between loading and the *Ss*' access to water. While Wayner and Reimanis (1958) found that following hypertonic subcutaneous NaCl injections drinking was independent of delays up to four hours, this may or may not be true for water consumption after stomach loads. Wayner reported no data for urine, but we have shown that both urine volume and urine concentration are functions of load concentration. To the degree that an animal can excrete salt by concentrating its urine before access to water it might need to ingest less water (as was the case with some of the satiated load

[5] L. I. O'Kelly, R. C. Beck, and Lowell T. Crow.

animals in Experiment 2); and if stomach loading is used in behavioral experiments the consequences of delays between loading and drinking (or training and testing of performance) should be understood.

Method

The *S*s were 120 rats, divided into 24 subgroups of five each and averaging 378 grams in weight. A random groups factorial design was used, varying 2% body weight volumes of 0, 2, 4, and 6% NaCl with delays of 60, 90, 120, 150, 180, and 240 min. between loading and drinking. Each *S* was given the same load and delay twice, with a six-day recovery period after each load. The animals were delayed in the metabolism cages following loading; urine was collected and analyzed from the delay period, as well as the first and second halves of the 4-hr. drinking period.

Results

Water Intake

Figure 12 shows the total four-hour intake as a function of load concentration and delay. These data were taken from the first load day only, but the functions were essentially the same for the second load day (as was true for all of the variables), differing only in the overall magnitude of intake. An analysis of variance showed that load concentration, delay, and load day variables all exerted significant effects on intake far beyond the .001 level. The delay × concentration interaction effect was just significant at the .01 level.

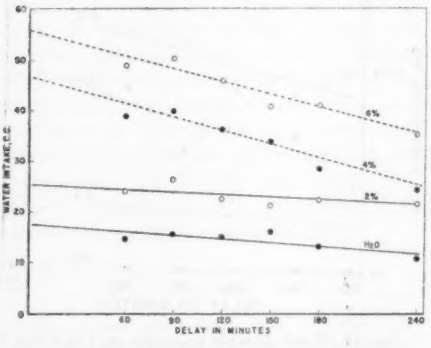

FIG. 12. Four-hour water intake on Load Day 1 as a function of delay of access to water following loading. (The lines are least squares fits to the points.)

The amount of water ingested in four hours showed a somewhat different relation between the load treatments than did either the 30-minute or 60-minute intake. Whereas the decline in consumption as a function of delay shows slightly after a half-hour of drinking and markedly after an hour, even at the 60-minute delay, the 4% and 6% groups drank the same amount in the first 30 minutes (cf. Figure 7). At neither 30 nor 60 minutes after access to water was there any separation between the intakes of the 2% and 4% NaCl groups delayed for four hours.

Weight and Drinking Changes on Interload Days

The changes in weight on the day following loading also declined as a function of delay, as did the 30-minute water intake on the day after loading. This, as in the previous experiments, seems to be a function of total intake on load days. The animals recover their weights and return to regular drinking well within the one-week recovery period, as was found in Experiment 3.

Urine Output

Figure 13 shows urine output during the delay intervals and for the total period of recording (delay plus drinking period). Urine output during the delay period proceeded at a constant rate whose slope was determined by load concentration, but total urine volume was very little affected by the length of the delay interval. The animals excreted about the same volume in five hours as in eight hours. To determine more specifically the effects of access to water on urination, the volume of excretion for the various subgroups was examined at 240 minutes after loading. At this time one group had not yet been given access to water and the other groups had been drinking for various times up to three hours. There were no differences among the six delay groups at each concentration, indicating that urine flow follows a course that

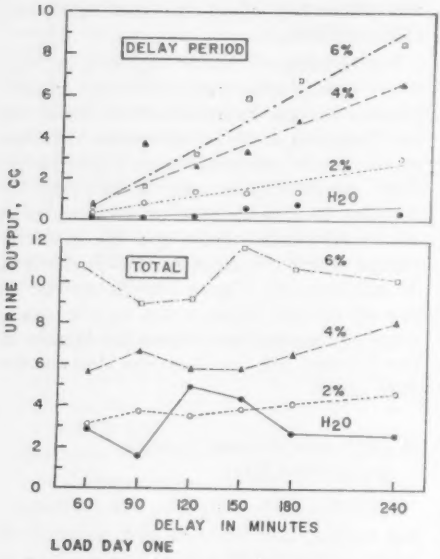

FIG. 13. Mean urine output during delay intervals on Load Day 1 (upper) and total urine volume (delay plus four-hour drinking period, lower).

is little, if any, influenced by whether the animal is drinking or not.

Urine concentration, on the other hand, is strongly affected by the animals' access to water. Chlorides, sodium, and potassium all increase in the urine as a function of delay, as well as of load concentration, as shown in Figure 14, which represents chloride excretion. The absolute amount of sodium and potassium excreted was less than that of chloride, but the functions were of much the same form. Concentration was highest during the delay period (about the same for all the NaCl load concentrations) and progressively declined during the first and second halves of the drinking period.

Discussion

The effects of delay on water intake, etc. are directly attributable to the fact that the *S*s excrete a more concentrated urine and hence a greater proportion of the ionic load if they do not have access to water. Drinking dilutes the fluid load so that it is more readily absorbed. Being diluted, the concentration differential between load and body fluids is lower and does not require as drastic an excretory correction.

These results become more meaningful when compared with those from Experiments 1 and 2. The total intake of the *S*s in those two experiments was the same, and urine volume was the same, but over the range of concentrations used in the present experiment the satiated *S*s put out a more concentrated urine in the last two hours than did the deprived. Since the deprived *S*s immediately drank a great deal more water after loading than did the satiated, the explanation of the urine concentration difference (whatever the exact mechanism may be) would seem to be the same as in the present experiment.

The data obtained in this experiment do not support the results of Wayner and Reimanis (1958), again probably due to differences in excretory control of the imposed loads. Since hypertonic solutions induce movement of body water toward them, in Wayner's experiment the water was initially moving toward the interstitial space under the skin of the back, whereas in our experiment the water went into the stomach and intestine. In this latter case, the absorptive surface is much greater, and corrective

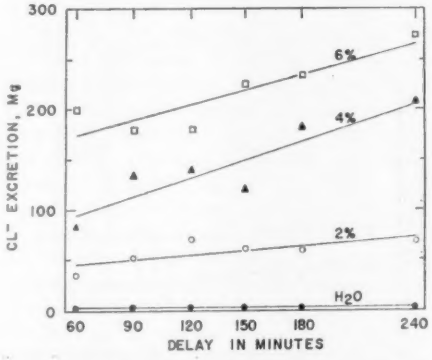

FIG. 14. Total chloride excretion on Load Day 1 as a function of delay. (Whereas urine volume was not necessarily increased by length of delay interval, chloride excretion clearly is for all loads except H_2O.)

filtration by the kidney would be more rapid.

In spite of the various changes that occur as a function of load-drink delay, the effects of concentration on drinking are maintained at all delays, with the exceptions of the 2% and 4% loads at the four-hour delay. With short delays there is no difference between the 4% and 6% loads in the first 30 to 60 minutes of drinking, but this is a temporary inhibition, not a differential management of the loads.

Experiment 5: A Further Study of Gastrointestinal Exchange Rates of Water and Sodium Chloride in Thirsty Rats[6]

In an earlier experiment (O'Kelly, et al, 1958) the gastrointestinal exchange rates of 3% body weight volumes of water and of 0.5, 0.87, and 3.0% NaCl were determined. The rates were studied up to a period of 35 minutes after loading. These rates were related to bar pressing following loads (O'Kelly & Falk, 1958) and to runway behavior (Solarz, 1958). Other experimenters have indicated the importance of post-ingestive factors in regulating drinking inhibition and other aspects of motivated behavior (Miller & Kesson, 1954; Miller, et al., 1957; Towbin, 1949). If we wish to control motivation by the "artificial" means presented in the previous experiments it is of some importance to know the rates at which the loads move through stomach and intestine and the amounts and distributions of the fluids accumulated in the gut by the osmotic action of the hypertonic loads. Accordingly, the purpose of this experiment was determination of gastrointestinal exchange rates for the 2% body weight volumes of water, 2.0, 4.0, and 6.0% NaCl used in the previous experiments.

Method

Subjects and procedure. The *Ss* were 285 male albino rats of the Holtzman strain, varying in age from about 120 to 200 days. All of them had been used in earlier experiments of various kinds, but were readapted to a 23.5-hr. deprivation schedule for at least 10 days before loading and gut determinations were made. The data were gradually collected over the course of a year as the animals became available.

The procedures are described in detail elsewhere (O'Kelly, et al, 1958). Animals are stomach loaded with measured volumes of fluid. After varying delays they are sacrificed, and the stomach, intestine, and cecum are clamped off and separately removed. The fluid content of each component is determined by obtaining its weight before and after oven drying to constant weight, with a correction made for inherent tissue water. From the measured volumes of fluid recovered from stomach, intestine, and cecum after the various delays, the rate of movement of fluid through these compartments may be determined.

Experimental design. The experiment called for 24 groups of 10 *Ss* each, given one of the four load concentrations at each of six delay periods: 15, 30, 60, 120, 180, and 240 min. Because the intestinal absorption curve for 6% NaCl had not reached an asymptotic level at 240 min., 5 animals with water loads and 10 animals with each of the NaCl loads were sacrificed after 300-min. delay. Additional 6% NaCl animals were run at 360 and 420 min., six at the former and four at the latter delay.

Results and Discussion

The results are summarized graphically, each point being the mean of all animals sacrificed under the particular delay interval indicated. Figure 15 shows the fluid recovered from the stomach after the various delays. Rate of fluid clearance is an orderly function of concentration. Water and 2% NaCl clear exponentially. After an initial period of stabilization at 70–90% of load

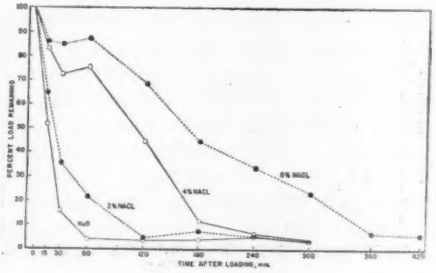

Fig. 15. Percent load volume remaining in stomach at various delays after loading.

[6] L. I. O'Kelly and R. C. Beck.

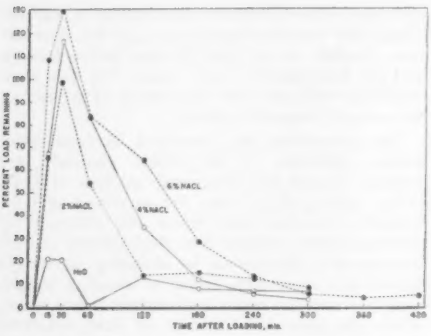

FIG. 16. Percent load volume remaining in the small intestine.

volume, the 4% and 6% NaCl solutions seem to clear in a similar fashion.

Figure 16 illustrates the time course of filling and emptying of the small intestine. Water manifests a small net gain of stomach clearance over absorption in the first 30 minutes after loading, but transport to cecum and absorption have emptied the small intestine of the water-loaded animals by 60 minutes. Accumulation of fluids from the hypertonic loads is, by 30 minutes, directly proportional to the NaCl concentration of the load, as is subsequent absorption and cecal flow. Intestinal distention by the hypertonic loads is maximal at 30 minutes and declines rapidly thereafter.

Accumulation and dissipation of fluid in the cecum is shown in Figure 17. Maximum fluid recovery occurred at 60 minutes post-loading, and cecal emptying is inversely proportional to load NaCl concentration. Since no diarrhea was observed during the delay periods, we assume that the major portion of the fluid remaining in the cecum is subsequently absorbed.

Figure 18 shows the percentage of load which is absorbed from the intestine at the several delays. For all the hypertonic loads there is an initial period of negative absorption for some time after loading—i.e., the amount of fluid in the intestine is greater than that given in the load. The rate of negative absorption is a direct function of load concentration, but the inflection point for all loads is at 30 minutes. The 6% load does not return to its initial volume until about three hours after loading, and 88% absorption is not achieved until seven hours after loading.

These curves suggest that in behavioral experiments it would be best to wait at least an hour, and preferably two hours after loading with hypertonic NaCl solutions before requiring the *Ss* to perform. Otherwise, the fluid content of the intestine will be rapidly increasing, with whatever effects this might have on performance—probably inhibiting. This problem will be discussed again after we have presented data from Experiment 6.

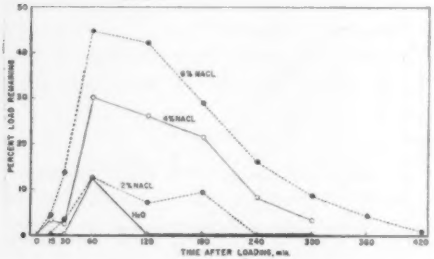

FIG. 17. Percent load volume remaining in the cecum.

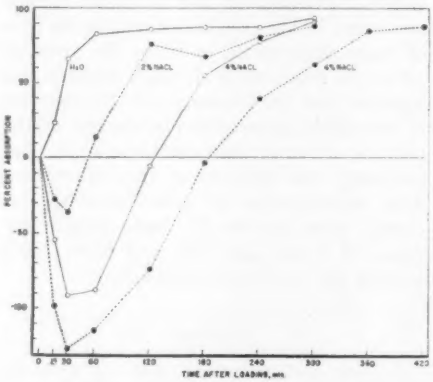

FIG. 18. Percent load volume absorbed at various delay times after loading. (Negative absorption indicates a recovery volume greater than the volume loaded.)

Experiment 6: Artificially Controlled Thirst and Water Reinforced Runway Behavior[7]

O'Kelly and Falk (1958) showed that when thirsty rats were loaded with water that water-reinforced bar pressing on a continuous reinforcement schedule was depressed in direct proportion to the load volume, and that it increased with various low concentration NaCl loads. Solarz (1958) found that running slows down as a water load has progressively more time to be absorbed before running is permitted. The present experiment was concerned with the problem: as a function of load concentration and runway length, how many highly massed, water-reinforced trials will a rat run in a single session? This question concerns both the effects of hydration and effort on performance and the methodology of using the loading technique in behavioral experiments.

Method

Subjects. The *S*s were 52 male albino rats of the Holtzman strain, 120-160 days of age at the beginning of the experiment. They had previously been used in other experiments, but had no prior experience with either runways or water deprivation.

Apparatus. A 2-ft. enclosed alleyway, to which a 4-ft. extension could be attached, was used. The delay between raising the start-box door and the *S* breaking a photocell beam 6 in. in front of the door (starting time) and the time between crossing the first beam and a second one 6 in. in front of the goal-box entrance (running time) were automatically recorded. When a water reinforcement of 40 licks was taken from the drinking tube at the back of the goal box, the tube was automatically withdrawn. The amount of water per lick was estimated from preliminary tests to be about .005 cc., or about 0.2 cc. per reinforcement.

Experimental design. Adaptation and runway training: The animals were adapted to a 23.5-hr. deprivation schedule and had two trials daily in the runway for a period of 10 days prior to testing. Each day they were weighed, run, watered for 30 min., then returned to the home cages where they lived in squads of four.

Test day: On Day 11 each member of a squad of four was loaded and after a 25-min. delay was run to its regular water reinforcement until it reached a criterion of three consecutive 60-sec. latencies in leaving the start box. If *S* did not leave the start box within 60 sec. or spent 60 sec. in the alley, he was removed and put into the goal box by *E* to determine if he would drink. Whenever *S* was in the goal box he was removed either just as soon as the tube was pulled out or after 60 sec. if he did not drink. Following removal from the goal box *S* was returned directly to the start box and the next trial was begun in about 15 sec., just enough time to allow *E* to record the times for the previous trial and to reset the clocks. When *S* reached criterion he was removed to the regular drinking box and allowed 60-70 min. of drinking.

For the next four days after testing the *S*s were maintained on the same 23.5-hr. deprivation schedule as during adaptation. On the fifth day they were loaded with the same solution as on the test day, and after a 30-min. delay were sacrificed. Their guts were processed as described in Experiment 5 (they provided the data for the 30-min. delay point in that experiment).

Results

Test Day Performance

Trials to criterion. Table 2 gives the number of trials to criterion for each of the *S*s. These are the number of times each *S* left the start box, whether or not he drank. An analysis of variance showed both the overall concentration variable and runway length to be significant beyond the .01 level of confidence. Individual comparisons of subgroups showed the significance of these functions to be due mainly to the relatively low scores of the H_2O and 6% groups, as opposed to the equally high scores of the 2% and 4% groups.

Water intake. On almost all trials when an animal drank in the goal box the full reinforcement was consumed, making it possible to plot the relationship between the amount of water received at the time the *S* reached criterion (load plus goal-box intake) and total intake (the hour's drink after criterion added). This is shown in Figure 19, which also has the number of reinforcements plotted on the right-hand ordinate. Whereas the function for goal-box intake is parabolic in form, total intake is a monotonic function with no significant differences between the two runway groups.

[7] R. C. Beck and L. I. O'Kelly.

TABLE 2

SCORES FOR TOTAL TRIALS RUN TO CRITERION

Runway Length		Load Concentration (% NaCl) 0	2	4	6
2 feet		7	40	55	5
		30	42	70	18
		30	55	80	25
		30	67	86	39
		31	79	90	141
		47	81	135	
			94		
	N	6	7	6	5
	M	29.2	65.4	86.0	45.6
	Md	30	67	83	25
	SD	11.7	19.1	24.7	48.9
6 feet		3	3	4	0
		6	10	4	5
		8	25	34	7
		12	42	46	9
		17	55	63	22
		29	78	84	37
		30	84	90	40
	N	7	7	7	7
	M	15.0	42.4	46.4	17.1
	Md	12	52	46	9
	SD	10.1	29.4	32.4	14.9

Note—All cell entries initially had seven *S*s but two were lost to illness, one died during loading, and one was discarded during training for repeated failure to run. The single lowest score for all concentrations with the 6-ft. runway were obtained in the same test session.

The two parabolic functions for number of reinforcements therefore reflect the effects of the different loads and runways on trials to criterion.

The goal-box drinking of the water and 6% groups was somewhat more affected than running; there were many trials when particular *S*s ran to the goal-box entrance but failed to drink. Thus, all the H_2O *S*s combined had a total of 46 runs without drinking and the 6% had 83, as opposed to 8 and 18 for the 2% and 4%. The running of the 6% *S*s was often very peculiar, reminiscent of a fraternity pledge pushing a peanut down the sidewalk with his nose. Such animals, in either runway, often ran to the goal-box entrance, then sat for 60 seconds until put into the goal box, where they would not drink. These same animals, on the other hand, drank immediately when put into the drinking box. The only apparent reason for this failure to drink in the runway was that the tube was slightly recessed, whereas in the drinking box the tube extended about a quarter of an inch into the box, making it more easily accessible.

The water-loaded animals had a slightly greater total intake on load day than on final adaptation day, 21.9 vs. 19.6 in the 2-foot group, and 21.6 vs. 20.7 in the 6-foot group. Considering that the animals had more time to drink on test day and that the goal-box intake was estimated, these correspondences are so close that we can quite accurately describe the total intake on load day as the amount of water needed to restore fluid balance. By inference we can describe the salt-loaded rats' intake in the same manner and by comparing intakes at the time of criterion with total intake we have the percentage of water needed which had actually been ingested. For the increasing concentrations of load these were: 2-foot: 58, 65, 63, and 31%; and 6-foot: 50,

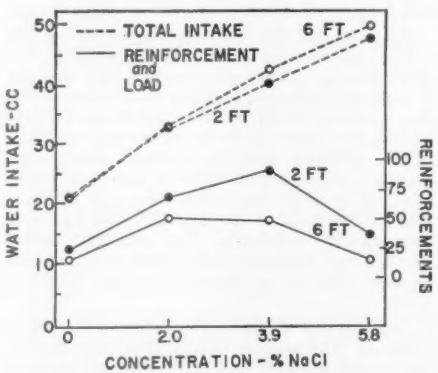

FIG. 19. Load day water intake and number of reinforcements. (The left ordinate is the volume of water consumed, and the right ordinate is the number of reinforcements, from which reinforcement intake is estimated. The upper curves are total intake on test days: load + reinforcement + water consumed during the hour post-session drinking period.)

51, 40, and 22%. The *S*s in the long runway were generally getting about 10% less of their total than were those in the short runway.

Time Scores

During training, the starting-time scores decreased steadily, but the animals in the 2-foot runway consistently started much faster than those in the 6-foot runway. Running times were about the same for both groups, when measured in feet per second. On load day the only differential effects of treatment on running, other than those due to differences in attaining criterion, were the slower starting times of the H_2O and 6% groups as compared with the 2% and 4% groups in the 2-foot runway (medians of the first five trials). On the day after loading, both starting and running times were significantly slower, but there was almost no differentiation among the variously treated subgroups and the animals in both runways increased about equally. Over the four post-load days these times gradually returned to their previous levels.

Weight and Drinking Changes

As found in the prior drinking experiments, there were increased weights and decreased drinking on the day after loading, but interestingly, the 6% groups had smaller changes than the 4% groups. This difference was significant at the .02 level for weight changes. The reason for this is not clear, but in the repeated load study, on the day after the first load, this occurred also. After the remaining five load days in that study, however, the 6% group did show the greatest change so the effect is still unclear.

Drinking was depressed following load day as a function of both concentration and runway length, rather surprising since both runway groups drank the same total amount on load day. This may, in fact, be no more than sampling error, though significant beyond the .01 level for both variables. The 2-foot group showed the least change.

Gastrointestinal Data

Since these data are the same as those in the clearance study, the reader may refer back to Figures 15–18. As indicated there, the amount of fluid in the stomach and intestine increases with the concentration of the load, and even with the 2% NaCl load the total fluid content of the stomach and intestine at 30-minutes post-loading exceeds the volume of the load. The gut data were correlated with the various running performances, but yielded no significant looking trends within the various subgroups. This lack of relationship may be for any of several reasons, such as the small number of *S*s per subgroup, low variability of scores for GI contents, or lack of reliability from one loading to another.

Discussion

Since the total water intake increased on the test day proportionally with load concentration, but trials to criterion did not, the loads are seen to have both inhibiting and facilitating effects. There are two aspects of the data to be accounted for: the differential proportion of total intake between the 2-foot and 6-foot groups at the time the criterion was reached, and the nonlinear relationship between trials to criterion and load concentration.

Two mechanisms conceivably were operative with respect to the difference between the two runway groups: differential conditioning and/or the differential effort involved in running the two alleys. Since the *S*s in the shorter alley consistently were faster in starting to run during training than were those in the longer alley, there was presumably some kind of differential conditioning such that a smaller amount of thirst reduction might suppress responding in the group with weaker conditioning. In combination with this, the inhibition developed from running the longer runway should tend to weaken the reaction potential for the running response more than would the reactive inhibition developing in the shorter alley.

The nonlinear results for trials to criterion may be explained by the inhibition from the load itself. The increasing amount of fluid in the gut following loading undoubtedly made running uncomfortable in the 6% groups and perhaps somewhat in the 4% groups. The relatively high incidence of running without drinking in the two extreme groups tends to be consistent with Solarz' report (1958) that, after water loading, running persisted longer than drinking. He discusses this finding in terms of differential excitability and suppression of habits by thirst "receptor" and "satiety" cues, drinking being more sensitive to changes in water deficit, increase or decrease, than running.

The present experiment controlled as much as possible the spacing of trials on test day and the delay between running and loading. These variables are interrelated insofar as either of them could allow differential changes in the transfer of fluid within the gastrointestinal tract which might in turn affect running significantly. The importance of the spacing of trials is indicated by comparing three animals loaded, respectively, with water, 2% and 4% NaCl and run in the 2-foot runway with trials separated by 90–120 seconds. Running the animals in rotation, the *E* extinguished after each had run 119 trials (compare with results in Table 2). A 6% animal ran only 16 trials, however, well within the range obtained in the experiment proper. The complex nature of the problem is further indicated by the 6% animal in the 2-foot group who ran 141 trials after loading. Either this *S* was not influenced by what we conceive to be the important inhibitory factors for the 6% group, or his stomach and intestine cleared the load in a very different fashion than did those of his running mates on load day. The amount of fluid in his GI tract following gutting, however, was just about at the mean of the 6% group.

The starting scores in this experiment were indicative of motivational effects induced by our loading, but the highly massed trials precluded precise information. It is probable that behavioral research using this technique of manipulating motivation should employ single trials, or some more optimal spacing. Since only one animal in the present experiment failed completely to run, there should be little attenuation from that source.

General Discussion

The Linear Scaling of Thirst

The results we have reported on facilitation of drinking by loads of hypertonic saline, when considered together with our findings of drinking inhibition by water loads varying in volume indicate the possibility of arranging load conditions in such a fashion as to produce a linear change in water consumption over a very wide range. If we may assume that drive strength in this situation is directly proportional to consummatory response, we see that stomach loading provides a technique for securing a linear scale of drive strength. It is of interest, therefore, to find a series of loads which will produce increments of the same slope, but opposite sign, as the H_2O decrements. Such a series is shown in Figure 20. The NaCl points were determined by obtaining a least squares fit to the H_2O points, then selecting values from the data of Experiment 1 which showed a reasonable fit to the upward extrapolation of the water-load regression. It turned out that increasing volumes of NaCl having about a 2% concentration potentiated drinking in 23.5-hour deprived rats to the same degree that the same volume of water depressed drinking. The 3, 4, and 5% volume points were obtained in Experiment 1; the 1 and 2% volume points were additional empirical checks.

The value of this particular exercise lies not so much in establishing the fact that drinking can be increased or decreased in a linear manner, since an infinite variety of loads might be used to give the same increments, but rather, that it can be done so easily and with such precision.

From the various experiments we can also summarize the results of loading with a variety of concentrations of 2% body

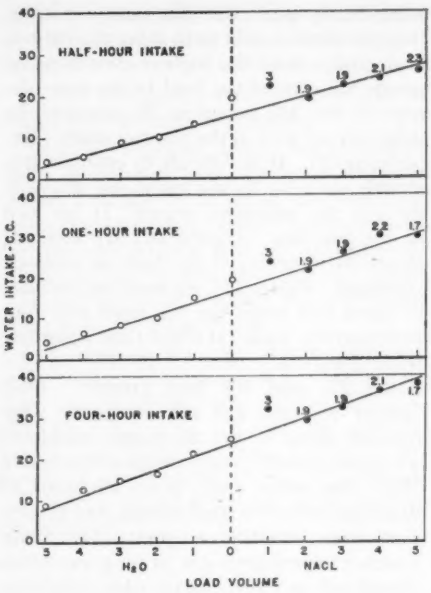

FIG. 20. Linear decrement and increment of drinking in thirsty rats. (The left half of the graph shows intakes at various times after different volumes of water loading—see also Fig. 3—and the right half shows intake after the same volumes of NaCl load. The exact concentrations of NaCl are shown with each point. The vertical dotted line goes through the zero-load points.)

weight volume of load, and compare this with the intake of water as a function of hours of deprivation. This comparison is shown in Figure 21. There is essentially a linear increase in drinking with load concentration up to 5% and then a sharp decline at 6%, on the 30-minute intake. The deprivation curve, on the other hand, is negatively accelerated; 47.5 hours of deprivation leads to the same 30-minute intake as does a load concentration of about 3% NaCl. An extrapolation from the deprivation intake curve to match the highest 30-minute intake after loading would require a number of hours of deprivation far beyond the limits of the rat; the four-hour intakes are totally beyond comprehension in terms of production by deprivation.

We may also refer back to Figure 20, and compare it with the deprivation curve in Figure 21. The highest NaCl load volume in Figure 20 (5%) produces a 30-minute intake which is just about the same as that produced by 47.5 hours of deprivation, but the former has the advantage that equal increments of volume produce equal increments of drinking, rather than progressively smaller increments. In addition, whereas the health of the 47.5-hour deprivation animals steadily declines over 20 days (as indicated by progressive weight loss), the loaded animals remain healthy and appear to thrive on the schedule (as shown, for example, in the weights and daily water intakes over the course of repeated loadings, as shown in Figure 11).

The Effect of Ether

One problem not considered in the experiments proper is the effect of ether per se on water intake, it having previously been shown to depress drinking in a 30-

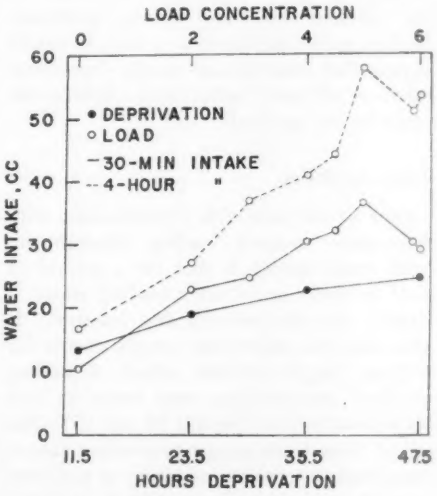

FIG. 21. Comparisons of water intake after varying hours of deprivation (the tenth deprivation interval on each schedule) with intakes following loading with various concentrations of 2% body weight NaCl. (The data for the 11.5-, 35.5-, and 47.5-hr. deprivation points were taken from an experiment run in our laboratory by Jack Dluhy on animals identical in age, sex, and strain to those in the present study.)

minute period (O'Kelly & Weiss, 1955). To estimate the results of etherization in Experiment 1, two additional groups of animals (with *N*s of 6 and 9, respectively) were tested for four-hour intake; one group had 23.5-hour deprivation, the other with deprivation followed by etherization before access to water. These are shown as the "0" load volumes in Figure 20. The etherized group had about 3 cc. less intake in 30 minutes than the nonetherized group, an intake falling directly on the regression line for tap water loads. The nonetherized group, on the other hand, fell above the regression. After four hours, however, these groups had the same intake, indicating that the ether effect had worn off. This is in agreement with the typical findings on humans, that ether is dissipated from the system almost completely in four hours (Goodman & Gilman, 1955, p. 63). Since the load groups and the etherized group all maintain their same relative positions throughout the four hours of drinking, and the no-load etherized group gradually catches up to the nonether group, it would appear that ether has an equally depressing effect on all loads, rather than a differential effect on any particular load.

Load Inhibition

One of the inevitable complications with hypertonic stomach loading (though not with water loads) is that for a period of half an hour or so after loading water is drawn into the stomach and intestine. It then does not clear these compartments for varying lengths of time, which, depending on load concentration, may range as high as several hours (Figures 15 and 16). Not all of these loads appear to produce gastrointestinal distentions inhibiting to performance, however, as witnessed by the fact that in the runway study the 2% and 4% animals did run a great deal more than the water-loaded *S*s. There may have been some inhibition with the 4% load, however, since the animals under that condition ran no more than those with the 2% load, even though they drank more. The 6% animals were clearly inhibited. The most conservative procedure would be to delay the various load groups until the highest concentration group had cleared the load to the same degree as the 2% group in 30 minutes, the delay period used in the runway study (Experiment 6). It is difficult to estimate this exactly since we do not know just *where* in the gut the inhibition occurs. If we look at the intestine (Figure 16) we estimate about 90 minutes; if we look at stomach clearance (Figure 15) we reach an estimate of about two hours for 4% loads and four hours for 6% loads (at which time, according to the delay study, there is little difference between 2% and 4% load groups). Only further research will tell us exactly what the best delay is, but all things considered we would presently estimate about two hours. With this period there is no inhibition of drinking in the 6% load groups, and absorption from intestine is pretty far along. Further experiments are in progress which should tell us more exactly what delays are best.

Summary and Conclusions

From all the experiments, the following results stand out:

1. With loading, one can control the water ingestion drinking of thirsty rats over a range from complete inhibition to an intake maximum unobtainable with deprivation.

2. The volume of a hypertonic NaCl stomach load plays a relatively small role in comparison with the amount of sodium chloride in the load. For a given amount of NaCl, the effect of increasing the volume of water is to reduce the intake to the degree that water has been given to the animal in the load itself.

3. Within the range of values studied intensively (0–6%, with 2% body weight volume), there was no harmful effect of the loads over half a dozen repeated loads or with long delays between loading and access to water. There is no reason to believe that this should not hold for all water loads we have used (1–5% body weight) or for all values of NaCl content which fall in this

range (i.e., absolute NaCl contents from 0.3 to 1.2 grams per kilogram of body weight, in solutions between 1–5% body weight).

4. The technique produces highly reliable results over repeated treatments.

5. With repeated loads, water intake tends to increase in a four-hour drinking period but this is offset by an increased urine output and decreased urine concentration such that the load is equilibrated to about the same extent over repeated loads. The increase in intake over repeated loads is the same for all loads used.

6. Following loading, weight increases and drinking decreases as a function of load concentration (and hence, of total intake on load day). A four-day recovery period is probably sufficient, but a six-day period is surely sufficient and is considerably more practical in terms of experimenter convenience.

7. As a function of delay of access to water following loading: (*a*) drinking in four hours decreases, (*b*) post-load weight and drinking changes are attenuated, (*c*) urinary excretion of ions increases, although total urine volume is about the same for all delay groups. The ability of the rat to manage salt loads by renal clearance is an important consideration and should be taken into account in any experiment with loads.

8. With the various hypertonic loads, water is drawn into the stomach and intestine for the first half-hour after loading. The time for clearance and absorption of the load is then an orderly function of load concentration. After loading with high concentration NaCl, drinking and performance are initially inhibited; a delay of one to two hours is suggested when stomach loading of hypertonic NaCl is used in behavioral experiments.

REFERENCES

BRUCE, R. H. A further study of the thirst drive with especial reference to the goal-gradient hypothesis. *J. gen. Psychol.*, 1937, **17**, 49–62.

BRUCE, R. H. The effect of lessening the drive upon performance by white rats in a maze. *J. comp. Psychol.*, 1938, **25**, 225–248.

GAMBLE, J. L. *Chemical anatomy, physiology and pathology of extracellular fluids.* Cambridge, Mass.: Harvard Univer. Press, 1958.

GILMAN, A. The relation between blood osmotic pressure, fluid intake and voluntary water intake. *Amer. J. Physiol.*, 1937, **120**, 323–328.

GOODMAN, L. S., & GILMAN, A. *The pharmacological basis of therapeutics.* (2nd ed.) New York: Macmillan, 1955.

HEYER, A. W., Jr. Studies in motivation and retention: III. A methodological approach to the independent manipulation of tissue dehydration and duration of water deprivation. *Comp. psychol. Monogr.*, 1951, **20**, 251–272.

HOLMES, J. H., & GREGERSEN, M. I. Observations on drinking induced by hypertonic solutions. *Amer. J. Physiol.*, 1950, **162**, 326–337.

MILLER, N. E., & KESSON, MARION L. Is distension of the stomach by a balloon rewarding or punishing? *Amer. Psychologist*, 1954, **9**, 430–431. (Abstract)

MILLER, N. E., SAMPLINER, R. I., & WOODROW, P. Thirst-reducing effects of water by stomach fistula vs. water by mouth measured by both a consummatory and an instrumental response. *J. comp. physiol. Psychol.* 1957, **50**, 1–5.

O'KELLY, L. I. The effects of preloads of water and sodium chloride on voluntary water intake of thirsty rats. *J. comp. physiol. Psychol.*, 1954, **47**, 7–13.

O'KELLY, L. I., & FALK, J. L. Water regulation in the rat: II. The effects of preloads of water and sodium chloride on the bar-pressing performance of thirsty rats. *J. comp. physiol. Psychol.*, 1958, **51**, 22–25.

O'Kelly, L. I., FALK, J. L., & FLINT, DOROTHY. Water regulation in the rat: I. Gastrointestinal exchange rates of water and sodium chloride in thirsty animals. *J. comp. physiol. Psychol.*, 1958, **51**, 16–21.

O'KELLY, L. I., & HEYER, A. W., JR. Studies in motivation and retention: V. The influence of need duration on retention of a maze habit. *Comp. psychol. Monogr.*, 1951, **20**, 287–301.

O'KELLY, L. I., & WEISS, H. H. The Effects of ether and a barbiturate on water regulation in the rat. *J. comp. physiol. Psychol.*, 1955, **48**, 123–125.

PFAFFMAN, C., YOUNG, P. T., DETHEIR, V. G., RICHTER, C. P., & STELLAR, E. The preparation of solutions for research in chemoreception and food acceptance. *J. comp. physiol. Psychol.*, 1954, **47**, 93–96.

SCHALES, O., & SCHALES, S. S. A simple and accurate method for the determination of chloride in biological fluids. *J. biol. Chem.*, 1941, **140**, 879.

SOLARZ, A. K. Effects of hydration on the running and drinking performance of thirsty rats. *J. comp. physiol. Psychol.*, 1958, **51**, 146–152.

TOWBIN, E. Gastric distention as a factor in the satiation of thirst in esophagatimized dogs. *Amer. J. Physiol.*, 1949, **159**, 533–541.

WAYNER, M. J. A study of the effects of sodium chloride and hydration on a monosynaptic reflex pathway in the spinal cord of the albino rat. Unpublished doctoral dissertation, University of Illinois, 1953.

WAYNER, M. J., & REIMANIS, G. Drinking in the rat induced by hypertonic saline. *J. comp. physiol. Psychol.*, 1958, **51**, 11–15.

(Received February 3, 1960)